Diurnal Patterns in Phytoplankton Photosynthesis, Fremantle Harbour

JEFFREY COSGROVE, NAVID MOHEIMANI & MICHAEL BOROWITZKA

Algae Research and Development Centre, School of Veterinary and Life Sciences, Murdoch University, South Street, Murdoch, WA 6150, Australia

✉ *J.Cosgrove@murdoch.edu.au*

Phytoplankton are likely to be one of the main sources of autochthonous production in the Swan Canning estuary yet only limited published data are available on their photosynthetic dynamics. Here we describe the phytoplankton community composition at the mouth of the estuary and diurnal pattern of phytoplankton photosynthesis by applying chlorophyll fluorescence techniques. Diatoms were the dominant taxa (46–88% of the phytoplankton community) recorded throughout the sampling period although their dominance appeared to increase with the flood tide. A diurnal pattern in photosynthetic performance was apparent via chl-*a* fluorescence measurements. Night-time deactivation of Calvin-Benson cycle enzymes was evidenced by low $rETR_{max}$ and light saturation point (E_k; minimum value of 207.5±2.3(se) µmol photon m^{-2} s^{-1}) values. Observed daytime increases in E_k (maximum value of 835.8±3.8(se) µmol photon m^{-2} s^{-1} at 4pm) with simultaneous reduction of alpha (α) suggests dynamic down-regulation of PSII electron transport during the day. Maximum quantum yield of PSII (F_v/F_m) decreased from a value of 0.81 to minima of 0.51 during high-light periods of the day. Accounting for these dynamics is important in the design of future studies of phytoplantkton productivity in the system. High recorded values of F_v/F_m and an unimpeded ability to photoregulate suggest the diatom-dominated marine phytoplankton community found in Perth's coastal waters at the time of this study was nutrient replete.

KEYWORDS: chlorophyll fluorescence, diurnal pattern, estuary, photoregulation, primary production, quantum yield

Abbreviations: α = intial slope of the photosynthesis vs irradiance curve; E_k = saturation irradiance; ETR = Electron Transport Rate; F′= chl-fluorescence level under actinic light; F_m = saturation pulse-induced maximum chl-fluorescence level from a dark-adapted sample when all photosystem II reaction centres are closed and non-photochemical quenching is negligible; F_m' = maximum chl-fluorescence level from a light-adapted sample exposed to a saturation pulse, closing all all photosystem II reaction centres; F_o = minimum chl-fluorescence level from a dark-adapted sample when all photosystem II reaction centres are open; F_v = maximum variable fluorescence (F_m-F_o); F_q' = variable fluorescence under actinic light (F_m'-F′); F_v/F_m = maximum quantum yield of photochemistry at photosystem II; F_q'/F_m = effective quantum yield of photochemistry at photosystem II in the light; RCII = photosystem II reaction centre; rETR = relative electron transport rate; $rETR^{max}$ = maximum relative electron transport rate.

INTRODUCTION

The city of Perth, Western Australia, surrounds the Swan-Canning Estuary. This microtidal, wave-dominated estuary is the second largest estuary in south-western Australia (Thompson 1998) and Fremantle Harbour is located at its mouth. Freshwater flow from tributaries is highly seasonal and a salt-wedge extends up the estuary from October to July/August (http://www.wrc.wa.gov.au/srt/riverscience/). The harbour is the transitional point between the oligotrophic coastal waters and the mesotrophic waters of the lower estuary.

Phytoplankton, microphytobenthos and seagrass are important sources of authochthanous production within the Swan-Canning Estuary (Hillman *et al.* 1995; Masini & Mccomb 2001) however there is a paucity of data on the photosynthetic dynamics of phytoplankton in the estuary and Perth coastal waters. While there have been some measurements of phytoplankton primary production (Thompson 1998), much of the literature has focused on the relationship between phytoplankton (chl-*a*, species composition) and environmental factors such as salinity, river flow, temperature and dissolved nutrients (Thompson 1998; Thompson 2001; Twomey & John 2001). Active chlorophyll fluorometry allows rapid estimation of photosynthetic parameters (e.g. quantum yield, electron transport rate) however the number of studies applying this technology to investigate diurnal patterns in phytoplankton photosynthesis *in situ* and with high sampling frequency is limited (Dijkman & Kromkamp 2006; Kurzbaum *et al.* 2010; Mackey *et al.* 2008; Verspecht 2007; Zhang *et al.* 2008).

Previous studies suggest that daily endogenous rhythms in the maximum rate of photosynthesis (P_{max}) and α occur in all major taxonomic groups of phytoplankton and that these rhythms are independent of changes in chl-*a* content (Harding *et al.* 1982; Boyd *et al.* 1997; Behrenfeld *et al.* 2004). Such rhythms should be taken into account when designing experiments investigation phytoplankton photosynthesis and interpreting results.

This study aims to describe the phytoplankton community present in Perth's coastal waters and investigate any diurnal pattern of photosynthesis by applying chlorophyll fluorescence techniques.

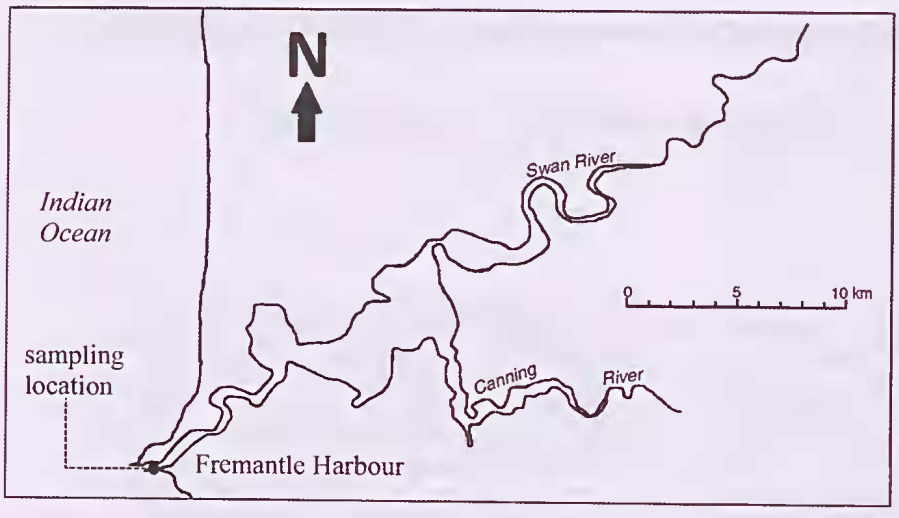

Figure 1. The study site on the seaward edge of Fremantle Harbour, the mouth of the Swan-Canning Estuary (modified from Hamilton *et al.* (2006)).

METHODS

Study site and environment

The study site was located at the mouth of Fremantle Harbour, with sample collection from a small motorised boat between the Maritime Museum of Western Australia and the OceanFarm jetty (Figure 1; GPS coordinates: 32°03'18.90" S 115°44'14.93" E). All sample collection and on-site measurements were performed on 7th December, 2005. Data on solar irradiance (W m^{-2}) incident at sea level were taken from Murdoch University meteorological station data (located 9.4 km from Fremantle Harbour; http://wwwmet.murdoch.edu.au/downloads.htm). These data were converted to photosynthetically available radiation (400–700 nm) in units of µmol quanta m^{-2} s^{-1} following the method of Thompson (1998). Tidal information was collected from the Coastal Data Centre (http://www.dpi.wa.gov.au/imarine/coastaldata/1895.asp) and on-site observations, while water surface temperature was recorded during each sampling occasion with a mercury thermometer.

Phytoplankton composition and chlorophyll

Samples for phytoplankton and chlorophyll were collected at 03:00 and every second hour until 19:00. A final sample was collected at 22:00 to yield a total of 10 samples for each analysis.

For phytoplankton identification and enumeration, at each sampling period a 1 L of near-surface (~0.5 m) seawater was collected from the end of the OceanFarm jetty into a thrice-rinsed Nalgene® bottle and 1 mL of Lugols iodine solution added. Analysis was performed using a Sedgewick-Rafter chamber at 200× magnification under a light microscope. Samples had previously been allowed to settle (>7 days) and concentrated 10–15 fold. Cell density calculation took concentration and dilution (due to addition of Lugols) into account. The most dominant phytoplankton were identified to species level using Tomas (1997), however in most instances cells were categorised into major taxonomic groupings only.

A further ~5 L near-surface (~0.5 m) grab sample was collected for chlorophyll-*a* determination. Samples were kept cool and in dim light until they could be returned to the university laboratory (no longer than 2 h) for filtration through Whatman GF/F (45 mm) at a pressure of 40±10 kPa. Filters were patted dry, wrapped in aluminium foil and frozen at -80°C for later extraction. Extraction was performed with ice-cold 90% (v/v) acetone by manual grinding using a glass mortar and pestle (modified from Strickland and Parsons (1972)). The homogenate was set in ice and away from light for 30 min and then clarified by centrifugation at 2100 rpm (1000 g) for 15 min in a Beckman GPR model centrifuge at 4°C. The centrifuge vials were then returned to ice and the supernatant used to determine chl-*a* content spectrophotometrically using the equations of Jeffrey and Humphrey (1975) for chromophyte algae containing chlorophylls *a*, c_1 and c_2. A Beckman DU-50 (UV-VIS) series spectrophotometer was used for all spectrophotometric measurements.

Chlorophyll fluorescence

Samples for chl-fluorescence measurements were collected from a small motorised boat using a 25 µm mesh-size phytoplankton net towed just below the surface until concentration of phytoplankton was sufficient to yield a satisfactory signal to noise ratio in the fluorometer. This generally took about 20–25 minutes. Upon return to the shore the suspension in the phytoplankton net cod-end was passed through a 180 µm mesh to remove zooplankton prior to sample measurements.

All fluorescence measurements were performed with a Water-PAM fluorometer (Walz, Germany) consisting of a Water-ED emitter-detector unit and a PAM-Control box. Data collection was via the WinControl (v2.08) software provided with the fluorometer. The fluorescence terminology used here follows that of Baker and Oxborough (2004). Samples were collected for fluorescence analysis on an hourly basis from 03:00 until 22:00 and were kept in dim light (shade) until measured.

Rapid light curves (RLCs) were performed to measure relative electron transport rate (rETR). Light levels were chosen, based on preliminary measurements (data not shown), to achieve a balance between maintaining as many points in the light-limited region of the curve while allowing an asymptote or down-turn to be evident. Gain levels were adjusted to maintain a dark fluorescence signal (i.e. measuring light only) of 200–300 units. Light

width at each irradiance was 5 s and saturation pulse duration was 0.8 s. For each sampling period ten replicate RLCs were performed on light adapted samples (kept in shaded conditions during collection procedure and during any delay before measurement). Ten replicate dark adapted F_v/F_m measurements were also collected at each sampling period. Dark-adaptation was for 15 minutes and samples were exposed to 5 s of far-red light immediately prior to applying the saturation pulse. rETR was calculated as (Cosgrove & Borowitzka 2010). Data were imported into Matlab® (Mathworks) and the model of Eilers and Peeters (1988) was fitted to the combined data (10 replicates) for each treatment to generate photosynthesis-irradiance (P-E) curve parameters. The error of the parameter estimates was derived from the covariance matrix of the fitted model and expressed as standard error.

RESULTS

Site conditions

Water temperature was 18°C during the early morning and late evening and 20°C throughout the day from 10:00 to 18:00. Lower temperatures coincided with ebb tide while higher temperatures occurred during flood tide.

Species composition, cell density and biomass (chl-a)

Diatoms (Bacillariophyceae) were found to be the dominant taxon throughout the sampling period. Diatoms were most dominant in the evening, representing a maximum of 88% of the phytoplankton community at 22:00 and a minimum of 46% at 15:00 (Figure 2). The highest percentage of diatoms occurred close to the end of the flood tide. The large species *Chaetoceros lorenzianus* and *C. curvisetus* were dominant at 15:00 while the small species (unidentified and referred to as *Chaetoceros* sp1.) was dominant in the evening (Table 1). The most commonly abundant species during the sample period were: *Chaetoceros* sp1., *Pseudonitzschia seriata*, unidentified nanoflagellates and *C. curvisetus*. Due to the predominance of diatoms throughout the entire sampling period it was assumed that changes in species composition had negligible impact on chl-fluorescence measurements.

The chl-*a* concentration of the marine waters at the mouth of Fremantle Harbour increased throughout the day, reaching a peak of 2.54 µg L^{-1} at 15:00 before decreased in the evening towards the end of flood tide. Average chl-*a* concentration across the entire sampling period was 1.73±0.18(se) µg L^{-1} (n=9). While phytoplankton cell density was generally positively related with chl-*a* concentration, there were some periods where dominance of large (i.e. 13:00) or small (i.e. 22:00) species yielded relatively high or low chl-*a* concentrations, respectively (r^2 shifts from 0.066 (n=9) to 0.592 with these two sampling times removed; data not shown).

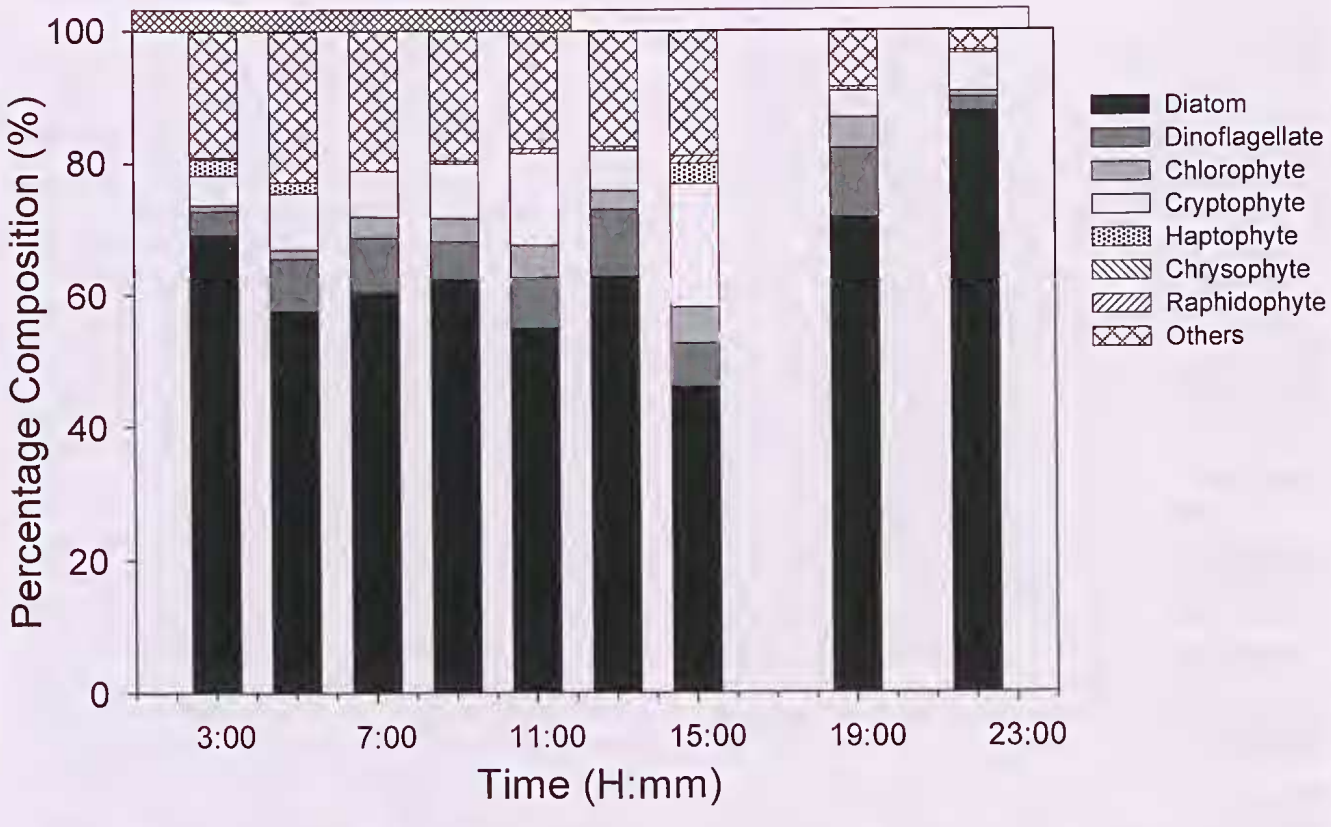

Figure 2. Percentage composition of phytoplankton taxa in Fremantle Harbour over the duration of the study period. Ebb tide is indicated by the thatched bar at the top of the graph while flood tide is indicated with an open bar.

Table 1. Fremantle Harbour phytoplankton community composition and relative abundance at ~0.5 m depth. Grab samples were collected every second hour throughout the day. (+) present, (++) present in moderate relative abundance, (+++) present at a high abundance relative to other species.

	3 AM	5 AM	7 AM	9 AM	11 AM	1 PM	3 PM	5 PM	7 PM	10 PM
Diatoms										
Bacillaria cf. *paradoxa*			+							
Cerataulina sp.	+						+		+	+
Chaetoceros curvisetus	+	+		+++	+++	+++	+++		+	+
Chaetoceros cf. *lorenzianus* var. *forceps*		+					+			
Chaetoceros cf. *laciniosus*			+		++	++	+++		++	
Chaetoceros decipiens				+	++	+			+	+
Chaetoceros tenuissimus						+				
Chaetoceros spl.	+++	+++	+++	+++	++	+++	+++		+++	+++
Cylindrotheca closterium	+	+	+	+	+	+	+		++	+
Cyclotella meneghiniana				+	+	+	+			+
Diploneis sp.			+	+						
Entomoneis sp.	+	+	+	+		+				
Guinardia sp.										
cf. *Gyrosigma* sp.						+	+			
Licmophora sp.					+				+	
Navicula spp.	+	+	+	+	+	+	+		+	+
Nitzschia spp.	+	+					+		+	
Pseudonitzschia seriata	+++	+++	+++	+++	++	++	++		++	+++
Pseudonitzschia pseudodelicatisima	+	+							+	+
cf. *Roicosigma* sp.						+				
Skeletonema costatum	++	+	+++	+		+++			+	+
cf. *Striatella* sp.									+	
Thalassionema sp.	+	+	+	+	++	++	+		+	+
Thalassiosira spp.			+						+	
Dinoflagellates										
Amphidinium sp.			+			+	+		+	
Ceratium furca	+		+			+	+		+	+
Dinophysis acuminata					+					
Diplopsalis sp.						+				
Gonyaulax sp.									+	
Gymnodinium spp.		+	+	+	+	+	+			+
Gyrodinium spp.	+	+	+	+	+	+	+			
Katodinium glaucum	+				+					
Katodinium rotundata		+	+		+	+	+		+	+
Polykrikos sp.									+	
Prorocentrum minimum		+	+		+	+	+		+	+
Prorocentrum micans			+	+	+	+	+			
Prorocentrum triestinum									+	+
Protoperidinium spp.	+		+		+	+			+	+
Scrippsiella cf. *trochoidea*	+	+					+		+	
Cryptophytes										
cf. *Plagioselmis* spp.		+	+	+	+	+	+		+	+
cf. *Rhodomonas* sp.					+					
cf. *Teleaulax* sp.	+		+			+	++		+	
Prasinophytes										
Pyramimonas spp.	+	+		+	+	+	+		+	+
Tetraselmis sp.		+	+	+	+		+		+	
Eugelenoids										
Eutreptiella sp.		+	+		+					
Chrysophytes										
Apedinella sp.	+	+								
Pseudopedinella sp.	+	+		+		+	+		+	+
Raphidophytes										
Heterosigma akashiwo	+				+		+			
Silicoflagellates										
Dictyocha fibula		+	+							+
Others										
Ebria tripartita							+			
Mesodinium rubrum	+	+	+			+	+		+	+
Nanoflagellates	++	++	++	+++	++	++	+++		+	+

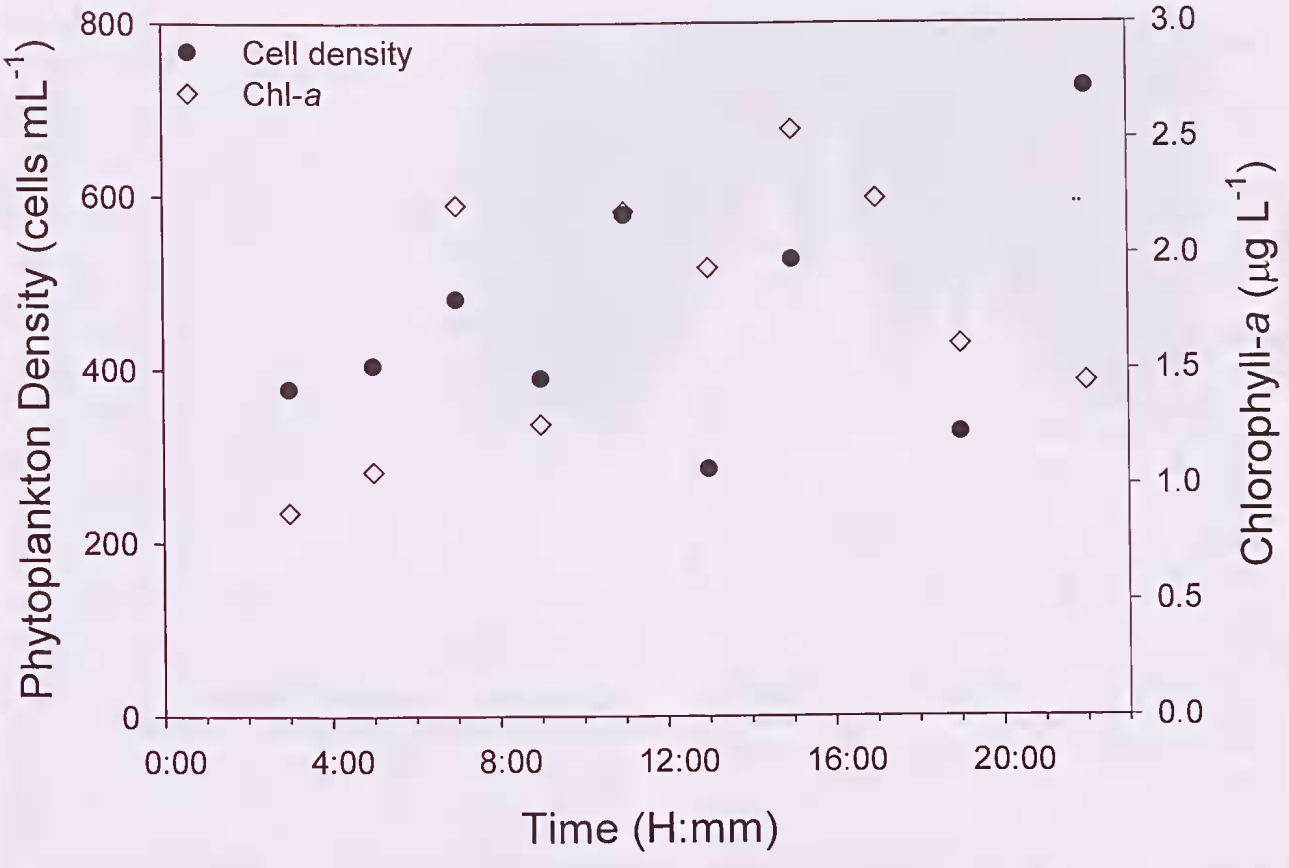

Figure 3. Phytoplankton cell density and chl-*a* concentration in Fremantle Harbour over the duration of the study period. Inconsistencies at 1300 hrs and 2200 hrs relate to changes in species composition.

Diurnal variability in photosynthesis

Analysis of F_v/F_m data revealed a typical diurnal cycle pattern (Figure 4a). F_v/F_m was quite high (~0.65) at 03:00 and 04:00, however immediately prior to dawn F_v/F_m increased to a value of 0.81 ± 0.01 (error = se, n = 10). This value was very close to theoretically maximal values of 0.83 (Magnusson 1997). A rapid decrease in F_v/F_m occurred over the early daylight hours and, throughout most of the day, values between 0.57 and 0.60 were observed. F_v/F_m dropped below this to values of 0.51 ± 0.02 at 09:00, 0.54 ± 0.01 at 13:00 and 0.51 ± 0.01 at 15:00. Each of these minima were associated with a period of high incident irradiance during the sample collection period (Figure 4a).

The maximum photosynthetic rates as measured by chl-fluorescence ($rETR_{max}$) indicate that photosynthetic processes were substantially down-regulated during the night (Figure 4b). Re-induction of these processes was rapid and possibly even predictive, since photosynthetic capability, as indicated by $rETR_{max}$, began to increase prior to dawn. This development of photosynthetic capacity was also reflected by the sudden and large increase in a (Figure 4c). $rETR_{max}$ reached a peak at 08:00 (408 ± 10.6 relative units, error = se, n = 10) before declining again as the first periods of high irradiance (852 µmol quanta m^{-2} s^{-1} at 08:30; Figure 4a) resulted in downregulation of photosynthesis and potentially some photoinhibition. Downregulation was indicated by the earlier and proportionately greater drop in a compared to $rETR_{max}$, leading to higher values for E_k (Figure 4d).

The depression of F_v/F_m at 09:00 and a concomitant decrease in $rETR_{max}$ and E_k, after a gap in the clouds resulted in the first period of high irradiance for the day (Figure 4), suggested photoinhibition due to damage of the photosynthetic apparatus may have occurred. A substantial recovery of F_v/F_m from 0.51 ± 0.02 (error = se) at 09:00 to 0.59 ± 0.01 in the following hour suggests that down-regulation rather than photoinhibition was responsible for the downturn in photosynthesis; however a continuing slow recovery of F_v/F_m over the next 2 hours of cloud cover (minima for this period was 318 µmol quanta m^{-2} s^{-1} at 10:50) indicates that some photodamage did occur. Depression of F_v/F_m was observed again at 13:00 and 15:00 after extended periods of high light, with the 15:00 depression being accompanied by decreases in $rETR_{max}$ and a despite slightly lower incident irradiances (Figure 4). By dusk $rETR_{max}$ began to decrease substantially despite large increases in photosynthetic efficiency (as indicated by α). This suggests substantial rate limitation by the Calvin-Benson cycle as its associated light activated enzymes begin to 'switch off'. The diurnal pattern of a closely reflected F_v/F_m changes, given that the initial slope is the product of the chl-specific absorption coefficient and maximum photosynthetic efficiency, this suggests that the light absorption characteristics remained relatively stable throughout the day.

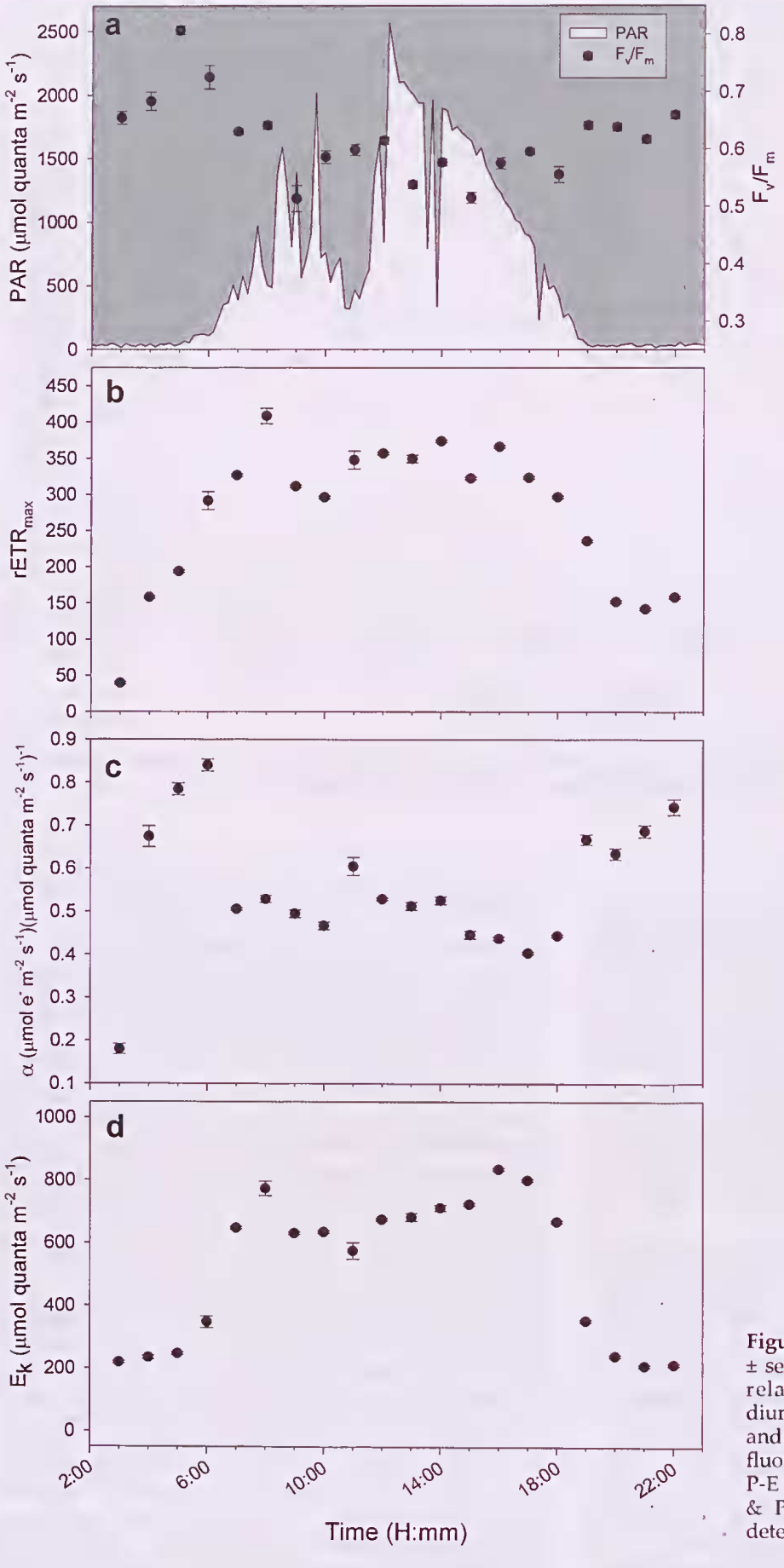

Figure 4. (a) Phytoplankton F_v/F_m (mean ± se, n = 10) over the course of the day relative to incident irradiance. The diurnal changes in (**b**) $rETR_{max}$, (**c**) alpha and (**d**) E_k parameters measured by chl-*a* fluorescence as determined by modelling P-E curve data with the model of Eilers & Peeters (1988) (error bars = se as determined by the model).

DISCUSSION

The phytoplankton community in the coastal marine waters sampled at the mouth of the Swan-Canning Estuary (this study) was found to be dominated by members of the Bacillariophyceae (diatoms) (Figure 2, Table 1). Dominance of diatoms in both near-shore coastal waters (Thompson & Waite 2003) and in the lower reaches of the Swan-Canning Estuary (Thompson 1998) has been described previously. The persistent dominance of diatoms throughout the study was fortunate, as shifts in taxonomic composition between species with different pigments (light harvesting and accessory) and/or thylakoid arrangements can influence the signature obtained by PAM fluorometers (Büchel & Wilhelm 1993). Thus, the observed taxonomic stability gave some confidence to the assumption that changes in fluorescence signal represented changes in physiological state of the phytoplankton community rather than taxonomic shifts.

Chl-*a* concentrations ranged between 0.88 µg L^{-1} at 03:00 to 2.54 µg L^{-1} at 15:00 (Figure 3). Peaks in chl-*a* concentration above ~1.6 µg L^{-1} were dependent on the presence of large, chlorophyll-rich diatom species such as *Chaetoceros curvicetus*, *Chaetoceros laciniosus* and *Skeletonema costatum*. The chl-*a* concentrations here were similar to those recorded at Blackwall Reach, about 6 km upstream, by Thompson (1998), although cell densities were substantially lower. Despite the Water-PAM's reported lower-limit of chl-*a* detection being 0.1 µg L^{-1}, the fluorescence signal was found to be unstable and sample concentration was required in order to achieve a stable and reliable fluorescence trace over the course of fluorescence measurements. Concentration of samples via the use of a plankton net may shock phytoplankton cells and can result in substantially lowered quantum yield measurements (Peter Ralph, pers. comm.). However, given that F_v/F_m values recorded after the concentration procedure were as high as 0.807 ± 0.007 (error = se, n = 10; Figure 4a), any negative impact on phytoplankton physiology was considered negligible.

Endogenous diel oscillations in the P-E response of marine phytoplankton have been widely reported (Boyd *et al.* 1997; Harding *et al.* 1981; Harding *et al.* 1982; Harris 1980) and diatoms appear to display greater diel periodicity than most other taxa (Behrenfeld *et al.* 2004; Harding *et al.* 1981). Studies have found maximum photosynthetic rate (P_{max}) to peak both in the morning (Marra *et al.* 1985) and afternoon (Harding *et al.* 1982). Timing differences in the pattern of diel changes have previously been explained by changes in latitudinal photoperiod and variations in nutrient assimilation and utilisation between phytoplankton size fractions (Harding *et al.* 1982). More generally, diel rhythms in P_{max} and α are commonly understood to result from oscillations between metabolic pathways which are influenced by light periodicity and exogenous nutrient supply (Harding *et al.* 1982; Behrenfeld *et al.* 2004). Nutrients accumulated during dark periods may help to yield a high P_{max} the following morning (Erga & Skjoldal 1990). Henley (1993) states that maximum photosynthetic rate usually peaks in the morning or close to midday and that this pattern may be endogenous, rather than related to chl-content, photoinhibition, nutrition or feedback inhibition by accumulated photosynthate. In this study, the observed pattern in $rETR_{max}$ appear to be a response to the changing light environment; with increasing values in the morning as key Calvin-Benson cycle enzyme such as Ribulose-1,5-bisphosphate-carboxylase/oxygenase (RuBisCo) become activated at dawn ("light activation") (Buchanan 1980) and down-regulation or inhibition during the middle part of the day as RCIIs become closed or damaged (Figure 4a, b). However, the significant increase in $rETR_{max}$ prior to dawn suggests that, to some extent, an endogenous rhythm was also present.

The PAM chl-fluorescence parameters F_v/F_m, $rETR_{max}$ and a indicate possible photoinhibition of phytoplankton photosynthesis at 09:00, 13:00 and 15:00 (Figure 4). Measurements at 09:00 were taken shortly after a break in cloud cover resulted in surface irradiances of ~1500 µmol quanta m^{-2} s^{-1} and F_v/F_m was found to be depressed (0.51 ± 0.023 compared to 0.64 ± 0.007 at 08:00). This drop in F_v/F_m was accompanied by a lower recorded $rETR_{max}$, while a had undergone a sudden decrease at 07:00 and subsequently remained relatively stable. A common measure of photosynthetic efficiency and physiological stress, the chl-fluorescence parameter F_v/F_m is extensively used to describe diurnal patterns in photosynthesis. Diurnal variability in F_v/F_m has been shown to be closely related to incident irradiance and midday depression of F_v/F_m is frequently observed when irradiance is high (ie. no or limited cloud cover) (e.g. Bergmann *et al.* 2002). However, it should not be assumed that F_v/F_m maxima and minima always occur during the evening and around midday respectively. Behrenfeld and Kolber (1999) describe measurements taken in the South Pacific Ocean (prokaryote-dominated phytoplankton community in iron limited conditions) that exhibit nocturnal F_v/F_m minima. Reduction of F_v/F_m can result from a number of physiological processes including numerous pathways for down-regulation of photosynthesis and closure of photosystem II reaction centres (RCIIs) due to photoinhibition associated with damage to the D1 protein within the core of photosystem II (Adams III *et al.* 2006; Cartaxana *et al.* 2013; Edelman & Mattoo 2006). Changes in chl-fluorescence can be used to make inferences about the photophysiological state of phytoplankton cells in the sample. A disproportionate reduction of F_o compared to F_m may represent enhanced xanthophyll cycle activity (photoprotection) or enhanced NADPH-reductase activity leading to state transition and photosystem II being in state-2 in the dark (Demmig & Björkman 1987; Magnusson 1997; Fracheboud 2001); while a decline in F_m and increase in F_o can indicate photoinhibition and closure of damaged reaction centres (Campbell *et al.* 2003; Franklin *et al.* 1992). The sampling methods undertaken did not allow for comparison of F_o and F_m between sampling periods and thus achieving a more quantitative assessment of the proportion of photoinhibition compared to photoregulatory down-regulation was not possible. The low signal to noise ratio of the conducted PAM chl-fluorescence measurements meant that quenching parameters were considered inconclusive (data not shown).

The highest irradiances for the day were recorded between midday and 13:00 as cloud cover that had persisted for most of the morning dissipated (Figure 4a). The F_v/F_m of the phytoplankton community decreased

from 0.61 ± 0.007 at midday to 0.54 ± 0.005 at 13:00 (Figure 4a), however on this occasion there was no concomitant decrease in $rETR_{max}$ (Figure 4b). Data from Olaizola *et al.* (1994) suggest that changes in diadinoxanthin cycle pigment pool size could occur between hourly sampling periods. An increase in dissipative diatoxanthin content relative to chl-*a* would result in a decrease in both F_m and F_o (Lavaud *et al.* 2004), however F_v/F_m would also decrease as the drop in F_m is proportionately greater. It has been proposed that epoxidase activity is inhibited after exposure to high light (Olaizola *et al.* 1994). Thus the low F_v/F_m recorded during this study at 13:00 may be the result of diatoxanthin remaining de-epoxidized in the dark and increased levels of non-photochemical quenching rather than significant photodamage to RCIIs.

Similar to the pattern seen at 09:00, at 15:00 the drop in F_v/F_m was associated with a concomitant reduction of $rETR_{max}$ indicating that some photoinhibition may have occurred (Figure 4a, b). However, substantial recovery of F_v/F_m was observed within the hour after each decline, indicating that that this was more likely to be an expression of dynamic regulatory mechanisms rather than photoinhibition (recovery from photoinhibition occurs on a time scale of hours to days).

Evidence of active quenching mechanisms was present in the pattern of E_k. Boyd *et al.* (1997) interpreted a significant decrease in E_k towards evening as relaxation of non-photochemical quenching. In this study, the substantially higher E_k values during the daylight hours compared to before dawn and after dusk (Figure 4d) resulted from a significant decline in α (Figure 4c) with little or no reduction in $rETR_{max}$ (Figure 4b) and can be construed as an increase in non-photochemical quenching. Also, the diurnal pattern of the P-E curve parameters tends to represent a photoacclimation response type A, as described by Richardson *et al.* (1983), where the activity of accessory pigments is thought to play a major role.

Diatoms, via the diadinoxanthin cycle, are capable of efficient downregulation of photosynthesis via non-radiative dissipation of excess energy (Cartaxana *et al.* 2013; Lavaud *et al.* 2002). The data presented here suggest that the diatom-dominated marine phytoplankton community found in Perth's coastal waters at the time of this study was capable of avoiding photoinhibitory damage by employing this tactic.

Along with the recorded high F_v/F_m values, the ability of the phytoplankton community to effectively regulate photosynthesis and avoid photodamage suggests that they were not nutrient limited. However, the nutrient concentrations of Perth's coastal waters have been described as "in the lower part of the range reported for temperate coastal waters elsewhere" (Johannes *et al.* 1994) with low N:P ratios resulting in a likelihood of N-limitation (Thompson & Hosja 1996). It is possible that phytoplankton at the mouth of the Swan-Canning Estuary at the time of this study were efficiently recycling nutrients from within the plankton community, or turbulence of the water column due water movement through the mouth of estuary provided sufficient nutrients via suspension of benthic solids however further studies would be needed to quantitatively determine phytoplankton nutrient status and the source of these nutrients.

CONCLUSION

The marine phytoplankton community at the mouth of the Swan-Canning estuary exhibited a typical diurnal pattern of photosynthesis, with maximum rates of photosynthesis occurring during the daylight hours as photosynthetic processes became rapidly induced. Studies assessing the productivity of microalgae in the system would need to take such diurnal variations into account.

High F_v/F_m values, reaching close to the theoretical maximum just prior to dawn, suggested that phytoplankton were physiologically competent and not nutrient-limited. The fast recovery of F_v/F_m after periods of high light while maintaining near maximal rates of photosynthesis ($rETR_{max}$) supports this finding and highlights the photoprotective ability of diatoms, which were the dominant taxon.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Andrew McMinn (University of Tasmania) for kindly lending a Water-PAM unit and the following people for assisting with sample and data collection in the field: Leah Barnes, Sam Bridgwood, Lara Centa, Sophie Fon Sing, Damian Priest, Rob Steuart, Luke Twomey, Florence Verspecht and Jason Webb.

REFERENCES

Adams III, W, Zarter, R, Mueh, K E, Amiard, V & Demmig-Adams, B 2006. Energy dissipation and photoinhibition: A continuum of photoprotection. *In*: Demmig-Adams, B, Adams III, W and Mattoo, A K (eds) *Photoprotection, photoinhibition, gene regulation, and environment*. Vol. 21. pp. 49–64. Springer, Dordrecht.

Baker, N R & Oxborough, K 2004. Chlorophyll fluorescence as a probe of photosynthetic productivity. *In*: Papageorgiou, G and Govindjee (eds) *Chorophyll a fluorescence: A signature of photosynthesis*. Vol. 19. pp. 65–82. Springer, Dordrecht.

Behrenfeld, M, Prasil, O, Babin, M & Bruyant, F 2004. In search of a physiological basis for covariations in light-limited and light-saturated photosynthesis. *Journal of Phycology* **40**, 4–25.

Bergmann, T, Richardson, T L, Paerl, H W, Pinckney, J L & Schofield, O 2002. Synergy of light and nutrients on the photosynthetic efficiency of phytoplankton populations from the Neuse River estuary, North Carolina. *Journal of Plankton Research* **24**, 923–933.

Boyd, P, Aiken, J & Kolber, Z 1997. Comparison of radiocarbon and fluorescence-based (pump-and-probe) measurements of phytoplankton photosynthetic characeristics in the northeast atlantic ocean. *Marine Ecology Progress Series* **149**, 215–226.

Buchanan, B B 1980. Role of light in the regulation of chloroplast enzymes. *Annual Review of Plant Physiology* **31**, 341–374.

Büchel, C & Wilhelm, C 1993. *In vivo* analysis of slow chlorophyll fluorescence induction kenetics in algae: Progress, problems and perspectives. *Photochemistry and Photobiology* **58**, 137–148.

Campbell, S, Miller, C, Steven, A & Stephens, A 2003. Photosynthetic responses of two temperate seagrasses across

a water quality gradient using chlorophyll fluorescence. *Journal of Experimental Marine Biology and Ecology* 291, 57–78.

Cartaxana, P, Dominques, N, Cruz, S, Jesus, B, Laviale, M, Serodio, J & Marques da Silva, J 2013. Photoinhibition in benthic diatoms assemblages under light stress. *Aquatic Microbial Ecology* 70, 87–92.

Cosgrove, J & Borowitzka, M 2010. Chlorophyll fluorescence terminology: An introduction. *In*: Suggett, D J, Prášil, O and Borowitzka, M A (eds) *Chlorophyll a fluorescence in aquatic sciences: Methods and applications*. pp. 1–17. Springer, Dordrecht.

Dijkman, N A & Kromkamp, J C 2006. Photosynthetic characteristics of the phytoplankton in the scheldt estuary: Community and single-cell fluorescence measurements. *European Journal of Phycology* 41, 425–434.

Eoelman, M & Mattoo, A K 2006. The d1 protein: Past and future perspectives. *In*: Demmig-Adams, B, Adams III, W W and Mattoo, A K (eds) *Photoprotection, photoinhibition, gene regulation, and environment*. Vol. 21. pp. 23–38. Springer, Dordrecht.

Eilers, P & Peeters, J 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological Modelling* 42, 199–215.

Erga, S R & Skjoldal, H R 1990. Diel variations in photosynthetic activity of summer phytoplankton in lindåspollene, western Norway. *Marine Ecology Progess Series* 65, 73–85.

Franklin, L, Levavasseur, G, Osmono, C B, Henley, W & Ramus, J 1992. Two components of onset and recovery during photoinhibition of *ulva rotundata*. *Planta* 186, 399–408.

Hamilton, D P, Douglas, G B, Adeney, J A & Radke, L C 2006. Seasonal changes in major ions, nutrients and chlorophyll *a* at two sites in the Swan River estuary, Western Australia. *Marine and Freshwater Research* 57, 803–815.

Harding, L W, Jr., Meeson, B W, Prézelin, B & Sweeney, B 1981. Diel periodicity of photosynthesis in marine phytoplankton. *Marine Biology* 61, 95–105.

Harding, L W, Jr., Prézelin, B B, Sweeney, B M & Cox, J L 1982. Diel oscillations of the photosynthesis-irradiance (p-i) relationship in natural assemblages of phytoplankton. *Marine Biology* 67, 167–178.

Harris, G P 1980. The measurement of photosynthesis in natural populations of phytoplankton. *In*: Morris, I (ed.) *Physiological ecology of phytoplankton*. pp. 129–187. University of California Press, Berkeley, CA.

Henley, W 1993. Measurement and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *Journal of Phycology* 29, 729–739.

Hillman, K, McComb, A J & Walker, D I 1995. The distribution, biomass and primary production of the seagrass *halophila ovalis* in the Swan/Canning estuary, Western Australia. *Aquatic Botany* 51, 1–54.

Jeffrey, S W & Humphrey, G F 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*, c_1, and c_2 in higher plants, algae, and natural phytoplankton. *Biochemie und Physiologie der Pflanzen* 167, 191–194.

Johannes, R, Pearce, A, Wiebe, W, Crosslano, C, Rimmer, D, Smith, D & Manning, C 1994. Nutrient characteristics of well-mixed coastal waters off Perth, Western Australia. *Estuarine, Coastal and Shelf Science* 39, 273–285.

Kurzbaum, E, Beer, S & Eckert, W 2010. Alterations in delayed and direct phytoplankton fluorescence in response to the diurnal light cycle. *Hydrobiologia* 639, 197–203.

Lavaud, J, Rousseau, B & Etienne, A-L 2004. General features of photoprotection by energy dissipation in planktonic diatoms (bacillariophyceae). *Journal of Phycology* 40, 130–137.

Lavaud, J, Rousseau, B, van Gorkom, H J & Etienne, A-L 2002. Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *phaeodactylum tricornutum*. *Plant Physiology* 129, 1398–1406.

Mackey, K R M, Paytan, A, Grossman, A R & Bailey, S 2008. A photosynthetic strategy for coping in a high-light, low-nutrient environment. *Limnology and Oceanography* 53, 900–913.

Magnusson, G 1997. Diurnal measurements of fv/fm used to improve productivity estimates in microalgae. *Marine Biology* 130, 203–208.

Marra, J, Heinemann, K & Landriau, G, Jr. 1985. Observed and predicted measurements of photosynthesis in a phytoplankton culture exposed to natural irradiance. *Marine Ecology Progress Series* 24, 43–50.

Masini, R J & McComb, A J 2001. Production by microphytobenthos in the Swan–Canning estuary. *Hydrological Processes* 15, 2519–2535.

Olaizola, M, La Roche, J, Kolber, Z & Falkowski, P 1994. Non-photochemical quenching and the diadinoxanthin cycle in a marine diatom. *Photosynthesis Research* 41, 357–370.

Richardson, K, Beardall, J & Raven, J A 1983. Adaptation of unicellular algae to irradiance: An analysis of strategies. *New Phytologist* 93, 157–191.

Strickland, J D H & Parsons, T R 1972. *A practical handbook of seawater analysis*. 2nd edn. Fisheries Research Board of Canada, Ottawa

Thompson, P 1998. Spatial and temporal patterns of factors influencing phytoplankton in a salt wedge estuary, the Swan River, Western Australia. *Estuaries* 21, 801–817.

Thompson, P & Hosja, W 1996. Nutrient limitation of phytoplankton in the upper Swan River estuary, Western Australia. *Marine and Freshwater Research* 47, 659–667.

Thompson, P & Waite, A 2003. Phytoplankton responses to wastewater discharges at two sites in Western Australia. *Marine and Freshwater Research* 54, 721–735.

Thompson, P A 2001. Temporal variability of phytoplankton in a salt wedge estuary, the Swan-Canning estuary, Western Australia. *Hydrological Processes* 15, 2617–2630.

Tomas, C R (Ed.) 1997. *Identifying marine phytoplankton*. Academic Press, San Diego.

Twomey, L J & John, J 2001. Effects of rainfall and salt-wedge movement on phytoplankton succession in the Swan-Canning estuary, Western Australia. *Hydrological Processes* 15, 2655–2669.

Verspecht, F 2007. *Temporal dynamics of the coastal water column*. Ph.D. Thesis, University of Western Australia, Perth.

Zhang, M, Kong, F, Wu, X & Xing, P 2008. Different photochemical responses of phytoplankters from the large shallow taihu lake of subtropical China in relation to light and mixing. *Hydrobiologia* 603, 267–278.